Oral Presentation (AQ-4)

Desinfection of Conveyance Using Bacteria Model (*Bacillus Subtillis* & *Bacillus Cereus*)

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INTRODUCTION

Indonesia is an agricultural country consisting of 17,504 islands, each of which has different biodiversity potentials. Increase in trade traffic today supported by the modernization of a fast-paced and sophisticated transpotation tool enables the spread of quarantine animal diseases from abroad into Indonesia, or across Indonesia.

A conveyance means used to transport animals or livestock from one area to another. This conveyance means in direct contact with the animals brought. The conveyance has the potential to transmit the disease from one animal to another and from one area to another. Animal transport means can be sea ships, cars, box cars, trucks, baskets etc.

Reducing the potential of disease transmission carried by conveyance, quarantine action is required on the means of conveyance. One of the measures against conveyance is disinfection. Disinfection is a process of reducing the number of disease-causing or potentially pathogenic microorganisms by physical and chemical. Disinfection is done using a variety of disinfectants that are easy to find and easy to use. The types of disinfectants commonly used in disinfection are iodine, alcohol, ammonium quartener, formaldehyde, potassium permanganate, phenol.

With this test, it is expected to get the type and effective disinfectant concentration to kill pathogenic microorganisms so that animal diseases transmission *from transportation can be minimized.*

To determine the type and concentration of effective disinfectants to kill pathogenic microorganisms especially spores that can be transmitted through a variety of conveyances.

MATERIALS AND METHODS

Materials and tools

Materials used in this study are Quartener Ammonium, Aceton Aquadest, Aquadest, *Bacillus Subtilis Bacillus*, bacterial growth media, basic material for transport (Wood, Plastic, Iron), and others. Equipment used are micro pipette, incubator, vortex, Biosafety cabinet class II, Sprayer, pickup car, basket, Ose, petri dish, test tube and other equipment.

Time and place

Implementation of the test of the disinfection of the instrument will be carried out at the Institute of Technical Test and Quarantine Method of Agriculture, Cibitung Bekasi and Field Application in BKP Kls II Cilegon, from January to November 2015.

Method

The test of disinfection of this conveyance is divided into 3 stages:

1. Preliminary Test
   - This test is also called disinfectant sensitivity test against bacterial spores of *Bacillus subtillis* and *Bacillus cereus*. The disinfectants are 6% formalin, 12% formalin, 5% glutaraldehyde, 10% glutaraldehyde, 5% ammonium quartener.
   - Disinfectant with bacterial spores *Bacillus subtillis* and *Bacillus cereus* spores mixed directly to obtain desired disinfectant concentration.
   - After 1 hour, 2 hours, and 4 hours are taken and planted in the media in order to see whether or not the growth of the bacterial spores.
2. Replication Test of conveyance.
   - Using the replica material of the conveyance of wood, plastic and metal.
   - The replica of this conveyance is placed above the surface with the size of 20 cm x 15 cm or 300 cm ^{2} which has been sterilized first.
   - Then contaminated with bacteria *Bacillus subtillis* and *Bacillus cereus* with soil and feces (to create conditions such as conveyance).
   - Infected by spray with concentration as in the preliminary test with the volume of each 75 ml tray.
   - After 1 hour, 2 hours, and 4 hours take swab of surface then planted in agar medium (Blood agar and nutrient broth) to be seen still grow or not the bacteria spores.

- The colonies in Nutrient broth (NB) after incubation is replanted in Blood agar in order to see if any bacteria are just injure (Faint), so after the nutrients can grow back.

3. Field Test
   - For field test, the transportation of wood and metal is used by pickup car, while plastic material is used animal basket.
   - The treatments were similar to replica tests, but without sterilization against the conveyance, nor the soil and feces.

RESULTS AND DISCUSSION

There are three stages of the test conducted the preliminary test, replica test of the means of conveyance, field test.

a. Introduction Test

In the Introduction test of several types and concentrations of disinfectant in the experiment, an effective disinfectant that can kill bacterial spores of *Bacillus cereus* and *Bacillus subtillis* is Formalin 6% and Glutaraldehid 5%. Further disinfectant with this concentration is used on the replica of a carrier made of metal, wood and plastic.

b. Replication test of conveyance

- The conveyance is made of metal

  In a replica test of a metal means of transport, Formalin 6% concentration has been able to kill bacterial spores of *Bacillus subtillis* and *Bacillus cereus*, while 5% gluraldehyde has not been able to kill both bacterial spores so that concentration is increased and an effective 10% glutraldehyde concentration is obtained. The existence of an effective concentration difference in glutraldehyde may be due to a reaction between disinfectants and metals or the occurrence of concentration dilution by the medium present above the metallic such as soil.
- The conveyance is made of wood

  For Wood-based transport vehicles with 6% formalin concentration and 5% glutraldehyde there is still growth of bacterial spores of *Bacillus cereus* and *Bacillus subtillis.* There is no new growth is absent in disinfection with Formalin disinfectant 8% and Glutraldehyde 10%. This is due to the material wood that has the ability to absorb the liquid so that in need of higher concentration to disinfect it.
- The conveyance is made of plastic

  The replica of a conveying device of concentrated plastic that can kill bacterial spores is 6% formalin and 10% glutraldehyde, of the effective concentration and type of disinfectant killing bacterial spores *Bacillus cereus* and *Bacillus subtillis are* used for field tests.

c. Field Test

After we have an effective concentrate disinfectant that kill bacterial spores of *Bacillus cereus* and *Bacillus subtillis* with replica test on conveyance, field test was conducted. From field test conducted in get data as follows

- Conveyance made of metal

From the statistical test, disinfection with 6% formalin and 10% glutaraldehyde had a marked effect on the destruction of bacterial spores of *Bacillus subtillis* and *Bacillus cereus*, but the duration of exposure time did not have a noticeable effect

Table1. Total Bacterial spores of *Bacillus cereus* and *Bacillus subtillis* on metals conveyances after disinfected

Desinfectan	Time (jam)	Bakteri (cfu/cm^2)	
Glutaraldehid 10%	0	448.3	A
	1	2.7	C
	2	0.3	C
	4	0.0	C
Formalin 6%	0	316.7	B
	1	0.3	C
	2	0.0	C
	4	0.0	C

- The Conveyance made of Wood

Disinfection with Formalin 8% and 10% Glutraldehyde on wood have a marked effect on the growth of bacterial spores but the duration of the exposure time does not have a noticeable effect. When compared between 8% formalin and 10% glutaraldehyde the ability to kill better bacterial spores is 8% formalin.

Table 2. Data of Spore *Bacillus subtillic* and *Bacillus cereus Bacillus* on Wooden conveyances after disinfection

Desinfectan	Time (jam)	Bacteria (cfu/cm^2)	
Glutaraldehid 10%	0	454.7	A
	1	1.3	C
	2	0.3	C
	4	0.0	C
Formalin 8%	0	281.3	B
	1	0.3	C
	2	0.0	C
	4	0.0	C

- The conveyances from Plastic

Table 3. Data of Spore *Count of Bacillus subtillic* and *Bacillus cereus Bacillu* on Plastics conveyance after disinfection

Desinfectan	Time (Jam)	Bacteria (cfu/cm²)	
Glutaraldehid 10%	0	304.0	A
	1	0.0	B
	2	0.0	B
	4	0.0	B
	1	0.0	B
Formalin 6%	0	289.3	A
	1	0.0	B
	2	0.0	B
	4	0.0	B

For Plastic materials between Formalin 6% with 10% glutraldehyid both provide a tangible effect to kill bacterial spores. Spores have some advantages compared in form cell vegetatif. As endurance to style mechanical, drought, radiation rays sun and high temperature. One spores that have endurance to temperature high is *Bacillus spores*, which make this spores could use as bioidicators sterilization and disinfection (Sugiono 2003, Dhirgo *et a*ll, 2007). Bacterial spores can survive hundreds or even millions of years in a state of inactivity. *Bacillus subtillic* spores can be killed by heating, irradiation, UV, and chemicals, due to damage to the structure of DNA. This treatment technique is very important in the food industry and medical products, to improve methods in killing bacterial spores effectively (Setlow, 2006).

Formalin and Glutaraldehid are derivatives of aldehyde derivatives, commonly used in water mixtures and denature bacterial cell protein (Siswandono, 1995; Palupi, 2005). In principle, these aldehyde derivatives can be used with wide spectrum. For example, formaldehyde kills microorganisms in rooms, utensils, and floors. The superiority of aldehyde derivatives is stable, persistent, biodegradable and compatible with some equipment materials. However, such compounds may lead to microorganism resistance, potentially as a carcinogen and cause irritation in the Kars mucosal system, 1995; Palupi, 2005). Because formaldehyde and glutraldehid can irritate the mucosal system, this disinfectant can only be used in conveyance after the animal is lowered.

CONCLUSION

a. Effective disinfectant for transport equipment made by metal is 6% formalin, wooden 8% formalin, and made from plastic is 10% glutraldehyde and 6% formalin.
b. The time of exposure showed no significant difference between 1 hour, 2 hours and 4 hours.

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